Neuropathological correlates of cumulative benzodiazepine and anticholinergic drug use

Authors:

Kathryn Richardson[a], Stephen B Wharton[b], Carlota M Grossi[a], Fiona E Matthews[c] Chris Fox[d], Ian Maidment[e], Yoon K Loke[d], Nicholas Steel[d], Antony Arthur[a], Phyo Kyaw Myint[f], Malaz Boustani[g], Noll Campbell[h], Louise Robinson[c], Carol Brayne[i], George M Savva[a,j]

Author affiliations:

a School of Health Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

b Sheffield Institute for Translational Neuropathology, University of Sheffield, Sheffield, S10 2HQ, UK

c Institute of Health and Society/Institute for Ageing, Newcastle University, Newcastle, NE4 5PL, UK

d Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ, UK

e School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK

f Institute of Applied Health Sciences, University of Aberdeen, Aberdeen, AB25 2ZD, UK

g School of Medicine, Indiana University, Indiana, 46202-3082, United States

h Department of Pharmacy Practice, Purdue University, West Lafayette, Indiana, 47907, US

i Cambridge Institute of Public Health, University of Cambridge, Cambridge, CB2 0SR, UK

j Quadram Institute Bioscience, Norwich Research Park, Norwich, NR4 7UA, UK

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Corresponding author: Kathryn Richardson, School of Health Sciences, University of East Anglia, Norwich, NR4 7TJ, UK, Email: Kathryn.richardson@uea.ac.uk, Tel: +44 1603 591070

Abstract

Background: Benzodiazepines and anticholinergic drugs have been implicated in causing cognitive decline and potentially increasing dementia risk. However, evidence for an association with neuropathology is limited.

Objective: to estimate the correlation between neuropathology at death and prior use of benzodiazepines and anticholinergic drugs.

Methods: We categorised 298 brain donors from the population-based Medical Research Council Cognitive Function and Ageing Study, according to their history of benzodiazepine (including Z-drugs) or anticholinergic medication (drugs scoring 3 on the Anticholinergic Cognitive Burden scale) use. We used logistic regression to compare dichotomised neuropathological features for those with and without history of benzodiazepine and anticholinergic drug use before dementia, adjusted for confounders.

Results: Forty-nine (16%) and 51 (17%) participants reported benzodiazepine and anticholinergic drug use. Alzheimer's disease neuropathologic change was similar whether or not exposed to either drug, for example 46% and 57% had intermediate/high levels among those with and without anticholinergic drug use. Although not significant after multiple testing adjustments, we estimated an odds ratio (OR) of 0.40 (95% confidence interval [95%CI] 0.18-0.87) for anticholinergic use and cortical atrophy. For benzodiazepine use, we estimated ORs of 4.63 (1.11-19.24) and 3.30 (1.02–10.68) for neuronal loss in the nucleus basalis and substantial nigra. There

was evidence of neuronal loss in the nucleus basalis with anticholinergic drug use, but the association reduced when adjusted for confounders.

Conclusions: We found no evidence that benzodiazepine or anticholinergic drug use is associated with typical pathological features of Alzheimer's disease, however we cannot rule out effects owing to small numbers.

INTRODUCTION

Drugs with anticholinergic activity (henceforth 'anticholinergics') block the neurotransmitter acetylcholine in the central or peripheral nervous system, affecting multiple body functions. Patients with a wide range of conditions such as urinary incontinence, Parkinson's disease, depression, epilepsy, gastrointestinal disorders and allergies may take drugs that have anticholinergic properties. Acetylcholine is strongly linked to learning and memory [1], and reductions in markers of the cholinergic system are found in both Alzheimer's Disease and Lewy body dementias, and correlate with cognitive decline [2]. Observational studies have suggested that long-term use of anticholinergic drugs increases the risk of developing dementia [3,4].

Benzodiazepines and related drugs including, zaleplon, zolpidem and zopiclone (referred to as Z-drugs) are commonly prescribed for anxiety and insomnia in older people. In the US, 9% of older adults currently use benzodiazepines, with 31% of these being long-term users [5]. Despite the well documented acute cognitive impairment associated with benzodiazepines, other side-effects including increased falls risk, tolerance and addiction, they are still used for long durations and at doses that exceed recommended limits [6]. Whilst earlier studies suggested long-term benzodiazepine use was associated with greater incidence of dementia, later studies do not [7–9].

Few studies have examined neuropathological correlates of long-term anticholinergic drug use. Increased Alzheimer's disease pathology (both amyloid plaque and neurofibrillary tangle densities) has been observed in Parkinson's disease patients with continuous use of anticholinergic drugs for at least 2 years [10]. Alzheimer's disease pathology, inflammation and other neurodegeneration have been linked to

anticholinergics in animal studies [11–13]. However a recent community-based autopsy study in humans reported no association between Alzheimer's disease-related neuropathological changes and anticholinergic use [14].

Studies examining the neuropathological consequences of benzodiazepine use are scarce [15]. An imaging study reported decreased amyloid load with chronic benzodiazepine use [15]. It is speculated that upregulating or preserving GABAAγ1/3 and γ2 receptors may protect neurons against neurofibrillary pathology in Alzheimer's Disease [16]. Theories vary as to whether benzodiazepine use may decrease or increase cognitive reserve [17,18]. In addition, benzodiazepines are considered to affect the α5-containing GABA(A) receptors, which are involved in cognition and preferentially located in the hippocampus [19].

In short, while there is some epidemiologic evidence of links between the use of anticholinergics and benzodiazepines with clinical outcomes such as subsequent cognitive decline and incident dementia [3,14,20,21], findings to date have been mixed, subject to potential biases and have little direct mechanistic support. In this study we test the hypothesis that the use of either class of drug in later life but prior to any dementia diagnosis is associated with the presence of neuropathologic features at autopsy, among a population representative cohort of older adults who agreed to brain donation upon death.

MATERIALS AND METHODS

The Medical Research Council Cognitive Function and Ageing Study (CFAS) is a multi-centre longitudinal population-based study of people aged 65 years and older in

England and Wales. The five centres (Cambridge, Gwynedd, Newcastle upon Tyne, Nottingham and Oxford) used identical methods to assess participants. Details of the study design have been described previously [22,23], but in short participants were selected at random from primary care lists in each of the five areas, and each was visited by an interviewer who recruited selected individuals into the study and conducted a baseline assessment. At baseline, trained interviewers completed a standardised questionnaire with participants that included sociodemographic and health questions and the Mini-mental State Examination (MMSE) [24]. A stratified sample of 20% was selected for more detailed evaluations that included a further participant interview and an interview with an informant. The participant interview included the full GMS-AGECAT diagnostic algorithm (equivalent to that in the Diagnostic and Statistical Manual of Mental Disorders, third edition, revised [DSM-III-R]) [25], and the Cambridge Cognitive Examination (CAMCOG) which is part of the Cambridge Medical Examination for the Elderly (CAMDEX) interviews [26]. Respondents were re-interviewed bi-annually with some follow-ups annually. When needed, interviews were assisted by an informant.

A brain donation programme was incorporated into all centres and predominantly focused on the detailed assessment arm of the study. The donor cohort is similar to the main study sample in all respects, other than those selected for invitation were weighted towards the cognitively impaired [27]. A retrospective informant interview (RINI) was conducted to ascertain the cognitive and health status of brain donors in the period between the last scheduled CFAS interview and death. MRC CFAS received multi-centre research ethics committee approval (Ref: 05/MRE05/37).

Dementia Diagnosis

Dementia status during each interview was derived using the full AGECAT diagnostic algorithm, defined as an organicity rating of level 3 or above [25,28]. This is equivalent to dementia as diagnosed by DSM-III-R [25]. Dementia at death was classified including information from the survey interviews, as well as interviews with informants after the respondent's death when this was possible, and death certification [29].

Neuropathology

Post-mortem tissue was obtained from respondents who signed a Declaration of Intent (DOI) to donate their brain after death and among whom a successful donation was then made. The neuropathologic assessment was carried out by clinical neuropathologists over the course of the CFAS study and the methods have been described elsewhere [27,29]. Briefly, macroscopic lesions, including infarcts, and focal and global atrophy (coded as none, mild, moderate or severe) were assessed at brain dissection. Depending on the centre, portions of brain tissue (either samples or slices of hemi-brain) were flash frozen and the remained fixed in formalin and sampled into paraffin blocks for the production of sections for histological assessment. Samples from the four neocortical areas, cingulate, hippocampus, basal ganglia, midbrain, pons, medulla and cerebellum were assessed following the protocol of the Consortium to Establish a Registry of Alzheimer's Disease (CERAD) with modification to include additional neurodegenerative and vascular pathology (see www.cfas.ac.uk) [30].

Alzheimer's disease neuropathologic change was measured using the 'ABC' score and categorised into high/intermediate or not/low [31]. Plaques were assessed using the CERAD method [30], and the maximum cortical neuritic plaque score calculated.

The neuroanatomical spread of neurofibrillary tangles (NFTs) was assessed according to Braak NFT staging, the six stages of which allow the neuroanatomical extent of NFTs to be classified into entorhinal (stages I-II), limbic (III-IV) and isocortical (V-VI) stages [32]. Assessment of Aβ phase was performed according to the Thal scheme [33], but only in the Cambridge and Newcastle centres [34]. Cerebral amyloid angiopathy (CAA) in leptomeningeal and parenchymal vessels was also assessed as none, mild, moderate or severe in each of the brain areas.

Other measures including macro and microvascular disease were documented, as well as neuronal loss in the hippocampus, entorhinal cortex, nucleus basalis, substantia nigra and locus ceruleus noted as none, mild, moderate or severe. Microinfarcts were recorded as any assessed in brain areas of the cortex (frontal cortex, temporal cortex, parietal cortex, occipital cortex, cingulate cortex, insular cortex, hippocampus [level of lateral geniculate body], entorhinal cortex and amygdala, cerebellar cortex) and separately in the subcortex (basal ganglia, midbrain, pons and medulla) [35]. White matter lesions were semi-quantified using post mortem MRI in three brain slices, and scored using the Schelten's scoring scheme [36].

Neuropathological examination was completed blind to clinical and interview data. Inter-rater reliability assessed by circulation of macroscopic brain photographs and microscopic slides among contributing pathologists was acceptable (<5% with scores more than 1 grade difference) [37].

Drug exposure

At each interview, participants were asked about their current use of medication with the question: ‘‘Do you take any medicine, tablets or injections of any kind, either that you buy yourself or that are prescribed by your doctor?’’ Drug name, dose, and frequency were recorded for each reported medication. We classified whether participants reported use (“any” anticholinergic use) of a drug scoring 3 on the Anticholinergic Cognitive Burden scale [38], and whether they reported use at more than one interview (“recurrent” use) occurring before one year prior to a dementia diagnosis. Similarly, we classified participants as having any use or recurrent users of a benzodiazepine (including Z-drugs). Interviews with drug data assessed included: baseline, 1-year, 2-year, 3-year, 6-year, 8-year, 10-year, 12-year, 14-year and 16-year follow-up. The number of interviews a patient completed depended on when they died, were recruited, met the dementia diagnosis criteria, and whether they were in the assessment arm with more frequent interviews. Participants were included in the analysis if they completed at least two interviews before one year prior to dementia diagnosis or death.

Statistical Analysis

Participant characteristics were compared across those reporting or not reporting anticholinergic drug use and benzodiazepine use. Neuropathology measures categorised by severity were dichotomised into moderate/severe or not, and low brain weight was defined as 1254<kg for men and 1120<kg for women. Analyses were later preformed instead using sex-standardised brain weight (measured as a continuous variable), with no effect on the study findings (results not shown). The association between each dichotomised pathological feature with levels of anticholinergic and benzodiazepine use was estimated using logistic regression.

Initial models were adjusted only for adjusting for age at death and sex. Further multivariable models included both anticholinergic and benzodiazepine exposure and were also adjusted for education (≤9 or 10+ years), baseline comorbidity (stroke, diagnosed hypertension, depression/anxiety, Parkinson's disease, and asthma), baseline sleep problems, and number of medication interviews completed.

Ordinal logistic regression was similarly used to estimate ORs for greater Braak staging and categorised Thal phase (0, 1-2, 3, 4-5) according to drug exposure. The proportional odds assumption was tested using the Brant test.

All analyses were performed using Stata version 15.1. Statistical significance was defined as $p<0.05$ on a two-sided test. However, due to the many neuropathological outcomes tested, we also used the Benjamini-Hochberg procedure to estimate the critical p-value threshold in order to control the false discovery rate (i.e. the proportion of rejected null hypotheses that are incorrect rejections) to less than 20% [39].

RESULTS

MRC CFAS recruited 13,004 participants, among whom 401 brain donations were successfully made by August 2015, and of whom 337 had two or more interviews recording medication data before death. Of these, 298 completed at least two interviews at least one year before meeting the dementia diagnosis or death, hence were included in our study. The median (inter-quartile range) duration in the study was 9 (5-13) years and the mean (standard deviation) number of interviews with medication exposure recorded was 4.2 (2.0).

Of the 298 brain donors, 51 (17%) participants reported anticholinergic use at at least one interview and 35 (12%) reported recurrent use. Of these, 33 (11%) reported antidepressant use, 11 (4%) reported urologicals, 2 (<1%) reported antiparkinson drugs, 3 (1%) reported antipsychotics and 4 (1%) reported antihistamines. Forty nine (15%) participants reported any benzodiazepine or Z-drug use and 33 (11%) reported recurrent use. This included 46 (16%) participants reporting benzodiazepine use and 5 (2%) reporting Z-drug use. Of the 83 participants reporting either benzodiazepine or anticholinergic drug use, 17 (20%) participants reported both.

At death, the mean (SD) age of the brain donors was 86.2 (7.4) years, 175 (59%) were women, and 102 (34%) had dementia. Those reporting benzodiazepine use, anticholinergic drug use and no use were generally similar in terms of time between recruitment and death, and in the proportions dying with dementia, or reporting stroke, asthma and hypertension during interviews (Table 1). However participants reporting anticholinergic drug use were younger at death, had less education, and were more likely to have depression, anxiety or Parkinson's disease. Those reporting benzodiazepine use were more likely to be women and have depression or sleep problems.

Neuropathological correlates of anticholinergic drug use

In general, there was no evidence of any associations between the distribution of neuropathology and anticholinergic medication use. In terms of statistical significance, no associations between anticholinergic drug use and any neuropathological features were detected after taking into account the multiple features tested, at the revised

critical threshold of p<0.008 (table 2). However, our findings are consistent with a wide range of effect sizes for many of the variables tested.

With respect to Alzheimer's disease neuropathologic change, 117 of 207 (57%) non-users had intermediate/high levels at autopsy compared to 22 of 48 (46%) anticholinergic users. Mean Braak stage was also similar between those who never reported anticholinergics (mean [SD] = 2.6 [1.6]), those who reported anticholinergic use once (2.1 [1.7]) and those reporting use more than once (2.7 [1.6]).

Although not significant after accounting for multiple testing, fewer cases of cortical atrophy were observed in anticholinergic drug users compared to non-users (adjusted OR=0.40, 95% CI 0.18 - 0.87), and even fewer were observed for recurrent users (adjusted OR=0.26, 95% CI 0.09 - 0.70). Neuronal loss in the nucleus basalis was more common with anticholinergic drug use when only adjusted for age and sex (OR=3.52, 95% CI 1.03-12.05), but this associated reduced when fully adjusted for the covariates (OR=2.06, 95% CI 0.49-8.68). There were no associations with macroscopic or microscopic cerebrovascular disease and anticholinergic use.

Neuropathological correlates of benzodiazepine use

No neuropathological features were more common with benzodiazepine drug use history after taking into account the multiple features tested (table 3). Comparable levels of Alzheimer's disease neuropathologic change was observed between non-users (53% with intermediate/high levels) and benzodiazepine users (64% with intermediate/high levels) at autopsy. Although not significant after accounting for

multiple testing, we estimated adjusted ORs for benzodiazepine use and neuronal loss in the entorhinal cortex, nucleus basalis, and substantia nigra of 2.47 (95% CI 0.90 – 6.76), 4.63 (95% CI 1.11 – 19.24) and 3.30 (95% CI 1.02 – 10.68), which were similar or stronger for recurrent benzodiazepine use. There was also suggestion of reduced cortical microinfarcts with longer benzodiazepine use (adjusted OR for recurrent use =0.11, 95% CI 0.02-0.63).

DISCUSSION

We found no association between anticholinergic use and pathological features typical of Alzheimer's disease. However, our findings are internally consistent with a wide range of effect sizes for many of the variables tested. Although not significant after accounting for multiple testing, we observed evidence of less cortical atrophy with anticholinergic use and less cortical microinfarcts with benzodiazepine use. We also observed evidence of neuronal loss in the entorhinal cortex nucleus basalis and substantial nigra with benzodiazepine use.

Our findings are similar to that of the Adult Changes in Thought study in finding no association between recurrent anticholinergic use and Alzheimer's disease neuropathological lesions [14], unlike suggestions from previous studies [10][11,12]. Reduced total cortical volume and temporal lobe cortical thickness and greater lateral ventricle and inferior lateral ventricle volumes were observed in patients treated with anticholinergics in a US Alzheimer's Disease Neuroimaging Initiative (ADNI) study [40]. However, we found evidence of decreased cortical atrophy and no evidence of temporal lobe atrophy in patients treated with anticholinergics. Our cortical atrophy measure likely represents synaptic and (to a lesser extent) neuronal loss and is a non-

specific effect of pathologies damaging neurons. Macroscopic atrophy and brain weight likely reflect synaptic loss and unlike typical Alzheimer's Disease neuropathology remain associated with dementia across the late-life spectrum [29]. Our findings are internally consistent with evidence towards associations of fewer CAA, cortical atrophy, Alzheimer's Disease neuropathology, and greater brain weight with anticholinergic use [41]. Whilst the underlying mechanism is uncertain, they do not point towards greater Alzheimer's Disease neuropathology with higher levels of anticholinergic drug use.

Our findings are consistent with the US Baltimore Longitudinal Study of Aging study that reported no effect of strong anticholinergic drug use on cortical grey matter volume using MRI [42]. We found no association between anticholinergic use and reduced microinfarct burden, contrary to the Adult Changes in Thought study [14], but found evidence of reduced cortical microinfarct burden with greater benzodiazepine use. Findings from pathology studies may vary due to differences in populations and the small numbers of participants included, differences in outcome measurement (e.g. MRI during life or pathology at death) and the specific methods for neuropathology measurement and classification, medication exposure measurement (e.g. current use or prescription or cumulative past use, or definition of anticholinergic), and in analytic approach and control for confounding. Regional vulnerability in the hippocampus could also play a role in the discrepancies between the various pathological studies examining the relationship between long-term anticholinergics exposure and AD pathology, however we were unable to examine this in our dataset [43].

Our findings of potentially greater neuronal loss in the nucleus basalis with both anticholinergic and benzodiazepine use are of interest. The nucleus basalis is rich in acetylcholine and stimulates the cholinergic system of the neocortex [44]. Neuronal loss in this region is thought to occur in the early stages of Alzheimer's disease [45–47]. It is reasonable to believe anticholinergic drugs could contribute towards neuronal loss in the nucleus basalis, as studies have identified that these neurons are susceptible to other toxic agents such as cadmium, aluminium, nitric oxide and ethanol [48–50]. However, reverse causation might also underlie this relationship. Loss of stimulation to the cholinergic system has been reported to lead to the development of psychiatric and behavioral symptoms termed 'Cholinergic Deficiency Syndrome' including agitation, anxiety, apathy, delusions, hallucinations and irritability [51–53][44]. Given that the strongest associations were observed for anticholinergic antidepressants, antipsychotics and benzodiazepines and neuronal loss in the nucleus basalis (results not shown), it may be that these drugs were prescribed to treat the symptoms of 'Cholinergic Deficiency Syndrome'. Other studies suggest that volume loss in the basal forebrain cholinergic system leads to widespread cortical atrophy in MCI patients [54], however we did not observe widespread cortical atrophy in our study.

Our study does not support the hypothesis that anticholinergic drug use contributes towards Alzheimer's Disease neuropathological lesion formation. A potential mechanism is highlighted whereby these drugs could impact on early stages of Alzheimer's Disease development via neuronal loss in the basal forebrain. However, it is more likely that anticholinergic exposure leads to more accelerated cognitive aging rather than to greater Alzheimer's disease pathology. A review concluded that

despite limited human data, tauopathy mouse models indicate anticholinergic drugs may enhance neurodegeneration with enhanced neuroinflammation including microglial activation [55].

The study benefits from being population-based with a long data history available before autopsy. Brain donors were found generally representative of the main study, except for being weighted towards the cognitively impaired by design [27]. The method used for diagnosing dementia has been validated and is widely used [56,57]. Detailed information was available from the interviews allowing us to use multivariable statistical techniques to reduce confounding and account for various indications for the drugs.

A major limitation of this study and other similar research based on brain banks is that our outcome measures can only be made at autopsy after death. Additionally, the cause and timing of death is likely to influence on the degree of pathology in the brain. To reduce this we adjusted for age at death and number of interviews, so that the effects of reported exposure are not confounded by the number of opportunities to report exposure, nor the age of the participant at the time of the outcome measurement. Although the neuropathological data was obtained using a standardised protocol and blind to any clinical information, we were limited by the historical measures available and future studies could benefit from more targeted measures. We assessed Alzheimer's diease neuropathologic change using the 'ABC' score and its component scores to reduce the impact of any interreater variability in plaque and tangle counts [31]. We were limited by not having Thal phase evaluated for all donors, however Thal phase is highly correlated with Braak

stage and neuritic plaque score and has been shown to provide little improvement in predicting dementia beyond these [34].

The small numbers of participants reporting anticholinergic and benzodiazepine use limited the statistical power of the study. None of the associations were statistically significant after correction to control the false discovery rate to less than 0.20. We lacked detailed information on drug exposure; for example, duration taking or prescribed the drugs was not recorded. Non-adherence is common is this population and we lacked data on drug adherence [58]. However, we are more confident that the participants took the drugs than in studies relying on prescription data, as use was self-reported and interviewers asked to see drug packages. Drug use was only recorded on the date of the intermittent surveys; hence we will have under-reported anticholinergic and benzodiazepine drug use. Misclassification of exposures in this way would be expected to weaken any associations detected. However, we tend to find good concordance in reports of drug use between successive assessments, suggesting that assessments are reasonably representative of medication use throughout the exposure period.

Our findings and those of others in this area highlight the limitations of current neuropathology databases for investigating the causes of brain changes, as well as suggesting important areas for future research. Further research is needed whether and how use of long-term drug use might lead to neuronal loss, or whether, as seems equally likely, drug use follows early changes in the brain which are only detected later on autopsy.

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Conflict of Interest/Disclosure Statement

The authors have no conflict of interest to report, except IM has received personal fees for guest lectures and to support travel from Astellas Pharmaceuticals, YL reports personal fees from Thame Pharmaceuticals, NC and CF have received grants and personal fees from Astellas Pharmaceuticals.

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Tables and Figures

Table 1. Characteristics of the brain donors by benzodiazepine and anticholinergic drug history

	Anticholinergic use (n=51)		No anticholinergic use (n=247)		Benzodiazepine use (n=49)		No benzodiazepine use (n=249)	
Baseline characteristics	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Women	33	64.7	142	57.5	36	73.5	139	55.8
Age at baseline, years*	73.8	7.5	77.2	6.8	79.3	7.8	76.1	6.8
Age at death, years*	84.7	7.1	86.7	7.3	87.7	6.8	85.9	7.3
Education, years*	9.4	1.7	9.9	2.1	9.7	1.7	9.8	2.1
Smoker	14	27.5	43	17.4	10	20.4	47	18.9
Duration in study, years*	9.7	5.8	9.1	4.6	9.1	5	9.2	4.9
Assessment arm	33	64.7	110	44.5	25	51	118	47.4
Centre								
Cambridge	17	33.3	59	23.9	18	36.7	58	23.3
Gwynedd	4	7.8	11	4.5	3	6.1	12	4.8
Newcastle	11	21.6	36	14.6	8	16.3	39	15.7
Nottingham	17	33.3	96	38.9	15	30.6	98	39.4
Oxford	2	3.9	45	18.2	5	10.2	42	16.9
Health conditions								
Depression	10	19.6	22	8.9	11	22.4	21	8.4
Anxiety	8	15.7	17	6.9	6	12.2	19	7.6
Asthma	9	17.6	22	8.9	6	12.2	25	10
Hypertension	23	45.1	87	35.2	14	28.6	96	38.6
Parkinson's disease	3	5.9	3	1.2	3	6.1	3	1.2
Stroke	7	13.7	21	8.5	5	10.2	23	9.2
Sleep problems	24	47.1	82	33.2	29	59.2	77	30.9
Duration of sleep problems, years^	9	4-23	8	4-15	11	6-24	7	4-14
Dementia at death	15	29.4	87	35.2	19	38.8	83	33.3

* mean (SD)
^ median (IQR)

Table 2. Adjusted odds ratios for anticholinergic drug use reported before dementia and neuropathology outcomes

Neuropathology outcome	Anticholinergic use, n(%)						OR (95% CI), age and sex adjusted		OR (95% CI), fully adjusted[a]	
	None (n=247)		Once (n=16)		Recurrent (n=35)		Any anticholinergic use	Recurrent anticholinergic use	Any anticholinergic use	Recurrent anticholinergic use
Low brain weight	84	34.1	3	18.8	8	23.5	0.59 (0.29 - 1.23)	0.62 (0.27 - 1.44)	0.55 (0.25 - 1.21)	0.51 (0.20 - 1.31)
Alzheimer's disease pathology										
Intermediate/high AD neuropathologic change	117	56.5	6	37.5	16	50.0	0.73 (0.38 - 1.40)	0.79 (0.37 - 1.68)	0.57 (0.27 - 1.20)	0.48 (0.20 - 1.19)
Cortical neuritic plaques[b]	68	28.2	5	31.3	7	22.6	0.89 (0.43 - 1.82)	0.73 (0.30 - 1.79)	0.85 (0.38 - 1.93)	0.71 (0.26 - 1.96)
Thal phase[c]	2.9	1.7	2.1	1.9	2.9	1.3	0.89 (0.38 - 2.05)	1.14 (0.42 - 3.07)	0.94 (0.37 - 2.42)	0.84 (0.26 - 2.72)
Braak NFT stage[c]	2.6	1.6	2.1	1.7	2.7	1.6	0.99 (0.55 - 1.77)	1.14 (0.57 - 2.27)	0.69 (0.37 - 1.31)	0.62 (0.28 - 1.37)
Congophilic amyloid angiopathy[b]	53	22.5	0	0.0	5	16.1	0.47 (0.18 - 1.27)	0.47 (0.18 - 1.27)	0.36 (0.12 - 1.05)	0.33 (0.11 – 1.01)
Neuronal loss/atrophy										
Neuronal loss[b]										
Hippocampus	21	8.8	2	12.5	1	3.3	0.87 (0.24 - 3.12)	NA	0.64 (0.16 - 2.59)	NA
Entorhinal cortex	26	10.9	2	12.5	3	10.0	1.13 (0.40 - 3.20)	0.95 (0.26 - 3.40)	0.89 (0.29 - 2.72)	0.55 (0.13 - 2.30)
Nucleus basalis	7	2.9	2	12.5	3	9.7	3.52 (1.03 - 12.05)	3.04 (0.72 - 12.79)	2.06 (0.49 - 8.68)	0.29 (0.03 - 3.06)
Substantia nigra	15	6.3	2	12.5	4	12.9	2.17 (0.78 - 6.03)	2.19 (0.67 - 7.15)	1.64 (0.43 - 6.29)	1.09 (0.20 - 5.91)
Locus ceruleus	12	5.0	2	12.5	0	0.0	0.83 (0.18 - 3.92)	NA	0.71 (0.11 - 4.65)	NA
Cortical atrophy										
Any	117	52.2	7	43.8	10	35.7	0.60 (0.30 - 1.18)	0.48 (0.21 - 1.11)	0.40 (0.18 - 0.87)	0.26 (0.09 - 0.70)
Temporal lobe atrophy[b]	36	16.4	4	25.0	1	3.7	0.82 (0.29 - 2.32)	NA	0.85 (0.26 - 2.80)	NA
Hippocampal atrophy[b]	38	17.3	2	12.5	1	3.7	0.41 (0.12 - 1.45)	NA	0.46 (0.12 - 1.80)	NA
Macroscopic cerebrovascular disease										
Atherosclerosis[d]	53	25.2	5	35.7	6	21.4	1.07 (0.50 - 2.30)	0.82 (0.31 - 2.14)	1.30 (0.54 - 3.09)	1.08 (0.36 - 3.21)
Infarcts	61	27.9	5	33.3	5	17.2	0.82 (0.38 - 1.78)	0.58 (0.21 - 1.61)	0.68 (0.28 - 1.65)	0.52 (0.16 - 1.64)
Lacunes	43	19.7	6	40	2	6.9	0.96 (0.41 - 2.25)	0.32 (0.07 - 1.39)	0.80 (0.31 - 2.09)	0.19 (0.04 - 1.00)
Microscopic cerebrovascular disease										
Atherosclerosis	67	29.6	3	20	6	20	0.59 (0.27 - 1.31)	0.58 (0.23 - 1.50)	0.59 (0.25 - 1.41)	0.75 (0.26 - 2.13)

Arteriolar Sclerosis	168	70.6	8	50	20	64.5	0.60 (0.31 - 1.18)	0.69 (0.31 - 1.54)	0.61 (0.28 - 1.31)	0.76 (0.30 - 1.95)
Cortical microinfarcts	44	25	2	15.4	6	27.3	1.12 (0.46 - 2.73)	1.52 (0.53 - 4.32)	1.24 (0.44 - 3.54)	2.69 (0.76 - 9.57)
Subcortical microinfarcts	35	19.9	3	23.1	4	18.2	1.13 (0.44 - 2.89)	1.00 (0.31 - 3.22)	0.95 (0.33 - 2.75)	0.90 (0.24 - 3.36)
White matter pallor	89	38.4	9	60	11	36.7	1.45 (0.74 - 2.81)	0.95 (0.43 - 2.12)	1.17 (0.56 - 2.43)	0.85 (0.34 - 2.10)
Deep white matter lesions[b]	76	45.5	5	38.5	6	37.5	0.66 (0.29 - 1.51)	0.63 (0.21 - 1.87)	0.47 (0.18 - 1.23)	0.30 (0.08 - 1.12)
Periventricular lesions[b]	57	37.3	4	33.3	5	31.3	1.40 (0.59 - 3.31)	1.28 (0.41 - 3.99)	0.82 (0.31 - 2.18)	0.68 (0.19 - 2.48)
Perivascular space expansion	170	71.1	12	75	22	71	0.88 (0.37 - 2.14)	0.79 (0.25 - 2.48)	0.94 (0.42 - 2.12)	0.70 (0.27 - 1.85)

a Adjusted for age, sex, stroke, diagnosed hypertension, depression/anxiety, asthma, Parkinson's disease, sleep problems, education, number of interviews, and benzodiazepine drug use.
b Rated as moderate or severe
c mean (SD)

Table 3. Adjusted odds ratios for benzodiazepine use reported before dementia and neuropathology outcomes

Neuropathology outcome	Benzodiazepine use, n(%)						OR (95% CI), age and sex adjusted		OR (95% CI), fully adjusted[a]	
	None (n=249)		Once (n=16)		Recurrent (n=33)		Any benzodiazepine use	Recurrent benzodiazepine use	Any benzodiazepine use	Recurrent benzodiazepine use
Low brain weight	76	30.8	4	25	15	45.5	1.34 (0.70 - 2.56)	1.73 (0.82 - 3.67)	1.53 (0.73 - 3.19)	2.34 (0.98 - 5.56)
Alzheimer's disease pathology										
Intermediate/high AD neuropathologic change	111	52.6	7	50.0	21	70.0	1.38 (0.69 - 2.76)	1.85 (0.79 - 4.35)	1.69 (0.75 - 3.80)	2.73 (1.00 - 7.50)
Cortical neuritic plaques[b]	62	25.7	5	33.3	13	40.6	1.72 (0.88 - 3.34)	1.88 (0.87 - 4.07)	1.88 (0.87 - 4.06)	1.94 (0.79 - 4.73)
Thal phase[c]	2.9	1.7	2.6	1.6	2.8	1.7	0.59 (0.26 - 1.32)	0.60 (0.24 - 1.54)	0.69 (0.25 - 1.88)	1.13 (0.36 - 3.55)
Braak NFT stage[c]	2.5	1.7	2.5	1.8	3.2	1.2	1.37 (0.76 - 2.46)	1.66 (0.85 - 3.27)	1.81 (0.93 - 3.53)	2.44 (1.12 - 5.31)
Congophilic amyloid angiopathy[b]	50	21.3	3	20	5	15.6	0.71 (0.31 - 1.65)	0.63 (0.23 - 1.75)	0.77 (0.29 - 1.99)	0.96 (0.30 - 3.08)
Neuronal loss/atrophy										
Neuronal loss[b]										
Hippocampus	19	8	1	7.1	4	12.5	1.19 (0.41 - 3.43)	1.34 (0.41 - 4.34)	1.25 (0.38 - 4.14)	1.85 (0.45 - 7.57)
Entorhinal cortex	22	9.2	1	7.1	8	25	2.09 (0.88 - 4.98)	2.82 (1.11 - 7.18)	2.47 (0.90 - 6.76)	3.98 (1.29 - 12.34)
Nucleus basalis	6	2.5	0	0	6	18.8	5.35 (1.59 - 18.03)	5.35 (1.59 - 18.03)	4.63 (1.11 - 19.24)	4.43 (1.06 - 18.46)
Substantia nigra	12	5	3	20	6	18.8	4.71 (1.80 - 12.31)	4.63 (1.55 - 13.83)	3.30 (1.02 - 10.68)	5.78 (1.43 - 23.39)
Locus ceruleus	11	4.6	1	6.7	2	6.3	1.46 (0.38 - 5.58)	1.35 (0.28 - 6.55)	1.44 (0.31 - 6.74)	2.10 (0.32 - 13.57)
Cortical atrophy										
Any	108	48.4	6	46.2	20	62.5	1.26 (0.65 - 2.45)	1.52 (0.70 - 3.31)	1.62 (0.74 - 3.54)	2.47 (0.98 - 6.23)
Temporal lobe atrophy[b]	29	13.3	2	15.4	10	31.3	2.01 (0.90 - 4.48)	2.36 (0.97 - 5.73)	2.51 (0.92 - 6.89)	2.85 (0.93 - 8.78)
Hippocampal atrophy[b]	32	14.7	0	0	9	28.1	1.15 (0.49 - 2.71)	1.15 (0.49 - 2.71)	2.01 (0.69 - 5.87)	1.94 (0.66 – 5.67)
Macroscopic cerebrovascular disease										
Atherosclerosis[d]	53	25.6	5	35.7	6	19.4	0.94 (0.44 - 2.01)	0.69 (0.27 - 1.81)	1.17 (0.48 - 2.85)	0.67 (0.21 - 2.08)
Infarcts	55	25.5	5	33.3	11	34.4	1.59 (0.79 - 3.18)	1.62 (0.72 - 3.65)	1.89 (0.82 - 4.32)	1.84 (0.69 - 4.89)
Lacunes	40	18.6	2	13.3	9	28.1	1.36 (0.63 - 2.94)	1.75 (0.74 - 4.15)	1.38 (0.56 - 3.44)	2.19 (0.74 - 6.45)
Microscopic cerebrovascular disease										
Atherosclerosis	60	26.8	7	46.7	9	28.1	1.34 (0.68 - 2.65)	1.00 (0.43 - 2.32)	1.49 (0.67 - 3.29)	1.01 (0.38 - 2.67)

Arteriolar Sclerosis	166	69.5	10	66.7	20	64.5	0.66 (0.33 - 1.32)	0.62 (0.27 - 1.40)	0.79 (0.36 - 1.74)	0.75 (0.29 - 1.95)
Cortical microinfarcts	46	26.6	4	36.4	2	7.4	0.53 (0.20 - 1.38)	0.22 (0.05 - 0.99)	0.35 (0.11 - 1.13)	0.11 (0.02 - 0.63)
Subcortical microinfarcts	35	20.2	3	27.3	4	14.8	0.86 (0.34 - 2.14)	0.65 (0.21 - 2.04)	0.86 (0.28 - 2.62)	0.59 (0.15 - 2.37)
White matter pallor	89	38.4	9	60	11	36.7	1.17 (0.61 - 2.27)	0.82 (0.37 - 1.85)	1.17 (0.55 - 2.46)	0.79 (0.31 - 1.98)
Deep white matter lesions[b]	69	42.9	4	44.4	14	53.8	1.40 (0.66 - 2.98)	1.56 (0.66 - 3.67)	1.38 (0.58 - 3.31)	1.85 (0.67 - 5.10)
Periventricular lesions[b]	54	36.5	3	33.3	9	37.5	1.54 (0.69 - 3.46)	1.58 (0.64 - 3.91)	0.89 (0.34 - 2.28)	0.91 (0.31 - 2.70)
Perivascular space expansion	170	71.1	9	60	25	78.1	0.91 (0.40 - 2.04)	0.94 (0.38 - 2.35)	0.91 (0.40 - 2.04)	1.30 (0.47 - 3.61)

a Adjusted for age, sex, stroke, diagnosed hypertension, depression/anxiety, asthma, Parkinson's disease, sleep problems, education, number of interviews, and anticholinergic drug use
b Rated as moderate or severe
c mean (SD)